

CLAIMS

1. A method of measuring the molecular mass of a compound Y of unknown molecular mass by mass spectrometry,  
5 comprising  
    providing a sample of compound Y,  
    providing samples of at least two different compounds each of formula (I), R-X in which R is a trityl group and X is cleavable to form a charged species for mass  
10 spectrometry,  
    and recording the molecular mass of compound Y and the at least two compounds of formula (I) in a mass spectrometer.
2. A method according to claim 1 in which R is  $R^1R^2R^3C$ - wherein  $R^1$ ,  $R^2$  and  $R^3$  are the same or different and each is  
15 a monocyclic or fused ring aromatic group that is substituted or unsubstituted.
3. A method according to claim 2 in which at least one of  $R^1$ ,  $R^2$  and  $R^3$  carries a substituent selected from  $C_1$ - $C_{20}$  alkoxy or hydrocarbyl, substituted or unsubstituted.
- 20 4. A method according to claim 3 in which the alkoxy or hydrocarbyl is substituted by carboxylic acid, sulphonic acid, nitro, cyano, hydroxyl, thiol, primary, secondary or tertiary amino, primary or secondary amido, anhydride, carbonyl halide or active ester.
- 25 5. A method according to claim 2 in which each of  $R^1$ ,  $R^2$  and  $R^3$  is aryl, preferably phenyl.
6. A method according to claim 1 in which the trityl group R includes two or four or more amide substituents.
7. A method according to claim 2 in which  $R^1$ ,  $R^2$  and  $R^3$   
30 together carry at least two amide groups and/or at least two reactive groups for coupling, preferably N-hydroxysuccinimide ester groups.
8. A method according to claim 1 in which X is halide or tosylate.
- 35 9. A method according to claim 1 comprising providing at least five, preferably at least ten compounds of formula

(I).and recording their molecular masses in a mass spectrometer.

10. A method according to claim 1 in which the group X is photocleavable to form a charged species for mass spectrometry.

11. A method according to claim 1 additionally comprising estimating the molecular mass of unknown compound Y as  $M_y$  and providing at least one compound of formula (I) which has known molecular mass  $M_1$  below  $M_y$  and at least one different compound of formula (I) which has molecular mass  $M_2$  above  $M_y$ , and preferably the difference between  $M_y$  and each of  $M_1$  and  $M_2$  is not more than  $\pm 50\%$

12. A method according to claim 1 additionally comprising providing a sample of at least one further compound Z of unknown molecular mass and measuring the molecular mass of compound Z.

13. Use of a compound of formula (I) R-X in which R is a trityl group and X is cleavable to form a charged species for mass spectrometry as a calibration compound for mass spectrometry.

14. A kit for the production of calibration compounds for mass spectrometry comprising:

(a) at least one base reactant of formula (I) R-X where R is a trityl group and X is cleavable to form a charged species for mass spectrometry and

(b) at least two different amine compounds which are of different molecular masses and which are each capable of reacting with the base reactant and base reactant (a) is packaged separately from amine compounds (b).

15. A kit according to claim 14, additionally comprising instructions to select at least two desired molecular masses  $M_1$  and  $M_2$  for the calibration compounds and to choose one or more amines for reaction with the base reactant so as to obtain compounds of the desired predetermined molecular masses  $M_1$  and  $M_2$ , and instructions to use the compounds in mass spectrometry.

16. A set of calibration compounds for mass spectrometry comprising at least two separately packaged mixtures (a) and (b), wherein

5 mixture (a) comprises at least two different compounds each of formula (I) R-X and having different molecular masses

10 mixture (b) comprises at least two further compounds of formula (I) R-X having different molecular masses and wherein R is a trityl group and X is cleavable to form a charged species for mass spectrometry.

17. A set according to claim 16 in which the lowest molecular mass in mixture (a) is lower than the lowest molecular mass in mixture (b) and the highest molecular mass in mixture (a) is lower than the highest molecular mass in mixture (b).

18. A set according to claim 16 in which each of mixtures (a) and (b) contains at least five different compounds of different molecular masses, preferably at least 10 different compounds of different molecular masses.

20 19. A set according to claim 16 comprising at least three separately packaged mixtures of compounds, preferably at least five separately packaged mixtures of compounds.

20. A kit for the production of a set of calibration compounds comprising a first package comprising a base reactant of formula (I) R-X, in which R is a trityl group and X is cleavable to form a charged species for mass spectrometry, and at least two separate second packages (a) and (b), each containing a mixture of at least two amine compounds which have different molecular masses and which  
30 are capable of reacting with the base reactant.

21. A kit according to claim 20 in which the lowest molecular mass in mixture (a) is lower than the lowest molecular mass in mixture (b) and the highest molecular mass in mixture (a) is lower than the highest molecular mass in mixture (b).

22. A kit according to claim 20 in which each of mixtures (a) and (b) contain at least five different amine compounds

of different molecular masses, preferably at least ten different amine compounds of different molecular masses.

23. A kit according to claim 20 comprising at least three mixtures of amine compounds, preferably at least five mixtures of amine compounds.

24. A method of measuring the molecular mass of a compound Y of unknown molecular mass comprising

estimating the expected molecular mass of compound Y, selecting at least one calibration compound of formula (I)

R-X having molecular weight close to the expected molecular weight of the compound Y, in which R is a trityl group and X is cleavable to form a charged species for mass spectrometry

and subjecting both compounds to mass spectrometry simultaneously.

25. A method according to claim 24 in which the calibration compound R-X is provided by selecting a base reactant R-X in which R is a trityl group different from R and selecting an amine reactant of appropriate molecular mass and reacting the amine reactant and the base reactant.

26. A mixture of at least two compounds of formula (I) R-X in which R is a trityl group and X is a group cleavable to give a charged species for analysis by mass spectrometry.

27. A mixture according to claim 26 comprising at least five, preferably at least ten different compounds of formula (I).

28. A method of mass spectrometry comprising subjecting simultaneously to mass spectrometry at least two different compounds of formula (I) R-X in which R is a trityl group and X is cleavable to give a charged species for analysis by mass spectrometry.